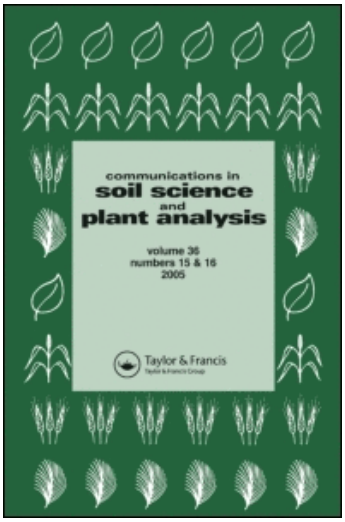

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Effect of Municipal Wastewater as a Wetland Water Source on Soil Microbial Activity

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Microbial activity levels of two soil materials, excavated from a wetland and irrigated with municipal wastewater effluent or Missouri River water, were compared. The wastewater had twice the electrical conductivity and four times the sodium concentration as river water. We performed activity assays on the soils before leaching, immediately after leaching, and after harvesting plants. Gas chromatography was used to measure carbon dioxide (CO_2) evolved in soil samples incubated for 7 d. Activity was significantly reduced in preleached wastewater–irrigated soils compared with river water–irrigated soils. Immediately after leaching, activity significantly increased and was similar to river water–irrigated soils. Activity decreased slightly after plant harvest in postleached treatments. Increased activity after leaching may be related to decreased salinity and sodicity, which probably lowered osmotic pressure in the soil. Our study demonstrated that soil salinity and sodicity induced by wastewater irrigation decreased microbial activity, which may impact nutrient cycling and glycophytic vegetation communities in wetlands.

Keywords Microbiology, salinity, sewage, soil water content

Introduction

Soil microbial activity is influenced by soil water content, illustrated by decreased aerobic activity when water-filled pore space of soil exceeds 60% (Linn and Doran 1984). However, microbial activity may be detrimentally affected before this soil water content is reached if water contains high salt concentrations (Pankhurst et al. 2001). Municipal wastewater effluent (WWE) containing high salt contents or Missouri River water (MOR) are used to irrigate wetland impoundments at the Eagle Bluffs Conservation Area (EBCA) located near McBain, Missouri (38° 53′ N, 92° 27′ W). We previously used soil materials collected from EBCA in a greenhouse study that examined seed bank response to repeated irrigation with WWE (Finocchiaro, Kremer, and Fredrickson 2009). In that study, salinity and sodicity rapidly increased in soil materials irrigated with WWE, which were responsible for inhibiting germination of the soil seed bank. Studies have shown seeds retrieved from soil often possess characteristic microbial associations (Kiewnick 1964; Kirkpatrick and Bazzazz 1979; Curl and Truelove 1986; Kremer 1993). Composition of the microbial

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associations may be affected by diffusion of antimicrobial substances secreted from the seed coat into the soil surrounding the spermosphere (McKey 1979; Kremer, Hughes, and Aldrich 1984; Kremer 1986; Kennedy 1998). These substances are thought to protect the seed from microbial decay. However, these microbial associations or physiological processes of the seed may become altered by soil salinity and sodicity (Baskin and Baskin 1998).

Microbial processes important for sustaining nutrient cycling also can be altered by salinity and sodicity. Several studies indicated soil microbial biomass, activities of various enzymes, and denitrification rates were enhanced by application of wastewater (Kannan and Oblisami 1990; Goyal, Chander, and Kapoor 1995; Monnett, Reneau, and Hagedom 1995; Filip, Kanazawa, and Berthelin 1999; Friedel et al. 2000). Filip, Kanazawa, and Berthelin (2000) reported that irrigation of a sandy Haplic Luvisol with municipal wastewater for almost 100 years resulted in increased microbial counts, total biomass, and enzyme activity and reduced C/N ratios compared with irrigation treatments free of wastewater. In contrast, other studies report that application of saline and sodic wastewater reduced microbial biomass, diversity, respiration, enzyme activity, and nutrient cycling (Ghinogeanu, Stephanic, and Jonescu-Sisesti 1984; Mahasneh, Budour, and Doddema 1984; Pankhurst et al. 2001).

Alterations to the plant community and nutrient cycles from irrigation with WWE may have prolonged and undesirable effects on the ecology of freshwater wetlands. Therefore, the effects of WWE as an irrigation source for wetlands should be understood to sustain these sensitive habitats. In this study, soil microbial activity determined by carbon dioxide evolution (CO_2) was compared in soil materials irrigated with either WWE or MOR. Because of the salinity and sodic concentration of the WWE, we hypothesized that soil materials irrigated with WWE will have less microbial activity than soils irrigated with MOR and decreasing salinity and that sodicity should increase activity.

Materials and Methods

Previous Treatment of Soil Materials

Soil materials were collected from an existing greenhouse study that consisted of large plastic microcosms (60 cm × 90 cm × 20 cm) filled with one of two soil materials and irrigated with either WWE or MOR to stimulate germination and vegetative growth of the soil seed bank (Finocchiaro, Kremer, and Fredrickson 2009). A greenhouse-treatment microcosm consisted of one soil material irrigated with one water source. For the present study, respiration assays were conducted on soil materials used in two sequential greenhouse trials separated by a leaching treatment. Each trial lasted approximately 100 d. At the beginning of each trial, microcosms were initially flooded with a water source until the volume of soil material was completely saturated and water ponding was evident (~5 cm). During trials, microcosms were not drained, and subsequent irrigations were applied to maintain soil water content of microcosms at approximately 80% field capacity for both soil materials. Water movement in microcosms was primarily influenced by evaporation and transpiration, which permitted soluble and insoluble constituents in the water sources to accumulate. At the end of each trial, all aboveground and belowground vegetation in the microcosms was harvested. In between trials, microcosms were leached to reduce the salinity and sodicity in the soil materials. Soil materials were flushed with deionized water, and powdered gypsum ($CaSO_4 \cdot 2H_2O$) was incorporated into the soil materials. Leaching was discontinued when the electrical conductivity (EC; 1:1 method; Whitney 1998) of the

soil materials was less than 3.5 mS cm^{-1}. Microcosms were not irrigated or disturbed in between trials except during the leaching treatment.

The soils used in the greenhouse microcosms were Sarpy loamy fine sand (mixed, mesic, Typic Udipsamments) and Blake silt loam (fine-silty mixed superactive, calcareous, mesic, Aquic Udifluvents), excavated from the 0- to 15-cm depth of the River Supply Channel at EBCA in June 1997. Soils of the River Supply Channel are inundated periodically throughout the year with MOR water and are not irrigated with WWE.

Soil Material Collection

Samples of soil materials were collected from microcosms at three different times. The first followed the first trial after vegetation was harvested from the microcosms (mid-November 2002) but before leaching (hereafter referred to as preleached). The second sampling was taken immediately after leaching concluded in early August 2003 (hereafter referred to as IAL). During the second sampling, seedling emergence was not apparent. The third was taken after the harvest of the subsequent trial in mid-November 2003 (hereafter referred to as postleached). At each sampling, a 5-cm diameter core sample of soil material was collected from the entire depth (0–15 cm), excluding the underlying gravel layer, of the same three replicates of each soil material irrigated with MOR or WWE. In addition, samples of the soil materials were collected from the River Supply Channel in mid-June 2003 and assayed separately from the greenhouse samples (field collected). Samples were passed through a 2-mm sieve and assayed within 72 h after collection with the exception of the field samples, which were stored at 0 °C for 9 d. Chemical analysis of the soil materials at the sampling times are listed in Table 1.

Microbial Activity

Microbial activity was estimated by measuring CO_2 evolution using the substrate-induced respiration assay (Horwath and Paul 1994). Five g of soil material (dry weight) were placed in glass tubes (10 cm × 1.5 cm) with screw caps fitted with septa. Soil material was adjusted to 18% moisture by weight with deionized water. Two hundred μL of 5% glucose solution was added to the moistened soil material in the tubes. Tubes containing the glucose-amended soil materials were immediately incubated at 27 °C in the dark for 24 h. Three tube replicates were prepared for each treatment replicate and for each field-collected soil material sample. Tube headspace contents were sampled for CO_2 at 24 h, 48 h, 72 h, and 7 d. A 1-mL sample was withdrawn from the tubes using a 1-mL syringe after aspirating the tubes five times. The 1-mL headspace volume from each tube was analyzed by gas chromatography (Buck Scientific model 910; PEAKNT software operating system) with a thermoconductivity detector (TCD) and He carrier gas at a flow rate of 14 mL L^{-1} using a silica-gel column at 50 °C. After each sampling, the caps of tubes were removed for approximately 5 min to allow ambient air to enter the tubes. Tubes were recapped and incubated after each sampling. Total CO_2 evolved over the 7-d period was determined from known calibration standards (Zibilske 1994).

Data Analyses

All values reported are expressed on a dry-weight basis after moisture content of assayed samples were determined from loss of weight after drying at 105 °C for 24 h. Treatment means were analyzed using repeated measures of samples and sampling time in an ANOVA

Table 1

Mean and standard deviation (given in parentheses) of total organic carbon (TOC), total nitrogen (TN), carbon to nitrogen ratio (C/N), electrical conductivity (EC), exchangeable sodium percentage (ESP), and pH (0.01 M $CaCl_2$) of soil materials irrigated with either Missouri River water (MOR) or municipal wastewater effluent (WWE)

		Silt loam						Loamy fine sand					
Sample period	N	TOC (%)	TN (%)	C/N	EC (mS/cm)	ESP (%)	pH	TOC (%)	TN (%)	C/N	EC (mS/cm)	ESP (%)	pH
Preleached													
MOR	3	0.80	0.073	11:1	5.3	19.1	7.6	0.26	0.029	9:1	12.4 (3.8)	40.2	7.8
		(0.01)	(0.003)		(3.8)	(5.1)	(0.06)	(0.01)	(0.009)			(5.1)	(0.06)
WWE	5	0.72	0.074	10:1	40.4	27.8	7.5	0.28	0.022	13:1	45.6 (2.9)	53.4	8.0
		(0.05)	(0.006)		(2.9)	(3.9)	(0.04)	(0.03)	(0.001)			(3.9)	(0.04)
IAL													
MOR	3	0.76	0.065	12:1	2.5	13.1	7.3	0.27	0.010	27:1	2.9 (0.5)	10.1	7.3
		(0.02)	(0.001)		(0.5)	(3.0)	(0.05)	(0.06)	(0.002)			(3.0)	(0.05)
WWE	3	0.73	0.062	12:1	2.6	26.5	7.5	0.20	0.011	19:1	3.2 (0.5)	16.6	7.4
		(0.03)	(0.001)		(0.5)	(3.0)	(0.05)	(0.04)	(0.003)			(3.0)	(0.05)
Postleached													
MOR	3	0.80	0.061	13:1	2.6	6.5	7.3	0.30	0.009	34:1	2.7 (0.2)	6.8	7.3
		(0.04)	(0.002)		(0.2)	(0.8)	(0.05)	(0.05)	(0.001)			(0.8)	(0.05)
WWE	3	0.77	0.061	13:1	4.2	14.1	7.5	0.24	0.008	29:1	3.8 (0.2)	11.1	7.4
		(0.02)	(0.004)		(0.2)	(0.8)	(0.05)	(0.01)	(0.002)			(0.8)	(0.05)
Field	2	0.92	0.083	11:1	0.23	0.9	7.27	0.70	0.071	10:1	0.22	0.9	7.29
		(0.13)	(0.018)		(0.03)		(0.06)	(0.02)			(0.01)		(0.06)

Notes. Soil materials were collected from Eagle Bluffs Conservation Area in June 2003 (field) and from greenhouse microcosms before leaching with deionized water (preleached), immediately after leaching (IAL), and after harvesting vegetation that germinated from the soil seed bank (postleached).

model using SAS (SAS Institute 2002–2003) procedure MIXED. Microbial activity (CO_2 evolution) data were analyzed by sampling period (i.e., preleached, IAL, postleached) with the total of 7 d accumulation as the dependent variable. Additionally, evolved CO_2 by incubation time was analyzed using a similar ANOVA model to possibly provide information on microbial groups (e.g., copiotrophs, oligotrophs). For all ANOVA models, a significance level of $P = 0.05$ was set to detect differences among treatment means. All mean separation analyses for ANOVA models used least squares means (LSM) comparison testing.

Results

Total CO_2 Evolution by Sampling Period

Prior to leaching, when soil EC was relatively high in WWE-irrigated materials (Table 1), CO_2 evolution was significantly greater in MOR-irrigated soil materials than in WWE-irrigated ones with respect to soil material ($F_{2,39} = 5.17$, $P = 0.0102$; Figure 1A). MOR-irrigated loamy fine sand had significantly greater CO_2 evolution than all other treatments ($P < 0.0004$); WWE-irrigated silt loam showed the lowest CO_2 evolution. Silt loam irrigated with MOR had similar CO_2 evolution as the WWE-irrigated loamy fine sand and field-collected, nonirrigated samples. Immediately after leaching, CO_2 evolution for MOR- and WWE-irrigated soil materials increased significantly ($P = 0.0021$, $P < 0.0001$, respectively) compared with preleached concentrations (Figure 2). The MOR- and WWE-irrigated soil materials sampled at IAL showed ~25% and ~45% increases in evolved CO_2, respectively, compared with preleached amounts (Figure 1B). At IAL, evolved CO_2 was similar within each soil regardless of water source, but both had significantly more than field-collected samples. After vegetative biomass harvest (postleach), evolved CO_2 from MOR-irrigated soil materials significantly declined by ~22% ($P = 0.0008$) relative to IAL amounts. Postleached CO_2 evolution of WWE-irrigated soil materials declined slightly (~6%) compared with IAL amounts. At this sampling, CO_2 evolution was similar in both WWE-irrigated soil materials and the MOR-irrigated loamy fine sand (Figure 1C). Carbon dioxide evolution from MOR-irrigated silt loam was significantly less than that of WWE-irrigated silt loam ($P = 0.0216$) and was similar to field-collected soil materials.

CO_2 Evolution by Incubation Time

Carbon dioxide evolution of preleached soil materials differed significantly for the combination of soil, water source, and incubation time ($F_{6,27} = 2.90$, $P = 0.0259$). No treatment differences were detected at 24 h of incubation; however, after 48 h, MOR-irrigated materials had greater CO_2 evolution than WWE-irrigated or field-collected soil materials (Figure 3A). In fact, MOR-irrigated loamy fine sand had significantly greater CO_2 evolution than all other treatments except MOR-irrigated silt loam ($P < 0.002$). After 72 h, MOR-irrigated loamy fine sand had significantly greater CO_2 evolution than all other treatments ($P < 0.001$), and CO_2 evolution of all other treatments were similar. After 7 d of incubation, the field-collected silt loam and loamy fine sand had the greatest CO_2 evolution and along with the WWE-irrigated loamy fine sand had significantly greater CO_2 evolution than other treatments ($P < 0.03$). Maximum evolution of CO_2 occurred at 48 h for MOR- and WWE-irrigated silt loam and at 72 h for MOR-irrigated loamy fine sand. Maximum CO_2 evolution occurred at 7 d for WWE-irrigated loamy fine and field-collected samples.

Carbon dioxide evolution of the treatments collected at IAL and postleached sampling periods were significantly different among water source and incubation time ($F_{6,27} = 7.36$,

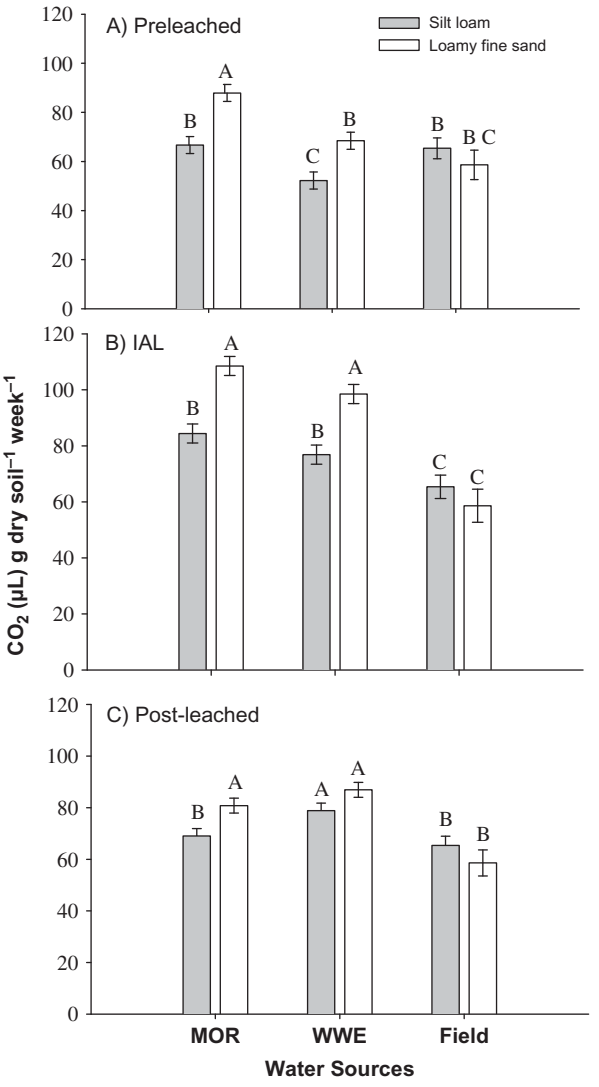

Figure 1. Mean total microbial CO_2 evolution of soil materials collected from greenhouse microcosms before leaching (preleached), immediately after leaching (IAL), and after leaching and harvesting vegetation (postleached) that were irrigated with Missouri River (MOR) or municipal wastewater effluent (WWE). Field-collected soils (field) were collected from a wetland impoundment at Eagle Bluffs Conservation Area. Vertical bars within columns indicate SE of the mean. Different letters among columns indicate means are significantly different ($P < 0.05$, LSM).

$P < 0.0001$; $F_{6,27} = 39.78$, $P < 0.0001$, respectively) but not between soil materials. Immediately after leaching, CO_2 evolution values between MOR-irrigated and WWE-irrigated soil materials were more similar than before leaching (Figure 3B). At 24 h, 72 h, and 7d of incubation, evolved CO_2 concentrations were not significantly different between the two water sources. Only after the 48 h sampling was CO_2 from WWE-irrigated soil materials significantly less than that of MOR-irrigated materials ($P = 0.024$). Maximum CO_2 evolution for MOR- and WWE-irrigated soil materials occurred after 48 h and

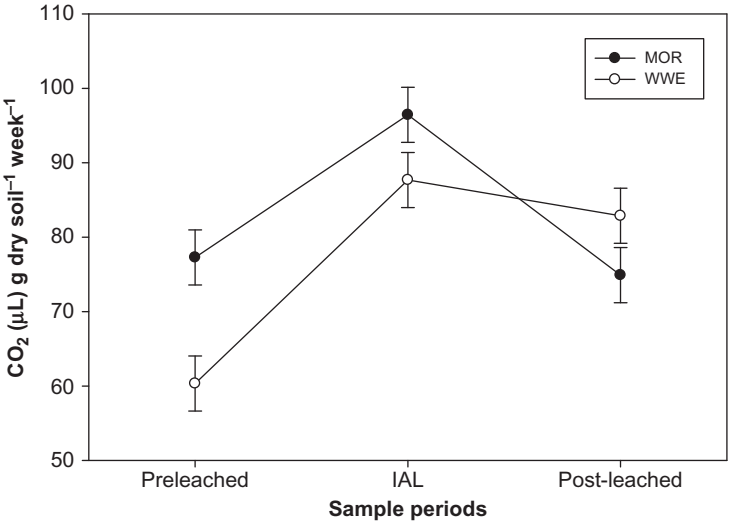

Figure 2. Mean total microbial CO_2 evolution for Missouri River–irrigated (MOR) and municipal wastewater effluent–irrigated (WWE) greenhouse microcosms regardless of soil material for all sample periods. Vertical bars indicate SE of the mean.

declined for each sampling thereafter. The exception was WWE-irrigated loamy fine sand, which had maximum CO_2 occurrence at 72 h. Postleached CO_2 evolution from WWE-irrigated soil materials was similar to MOR-irrigated materials for nearly all sampling times (Figure 3C). At 48 h, however, WWE-irrigated soil materials had significantly greater CO_2 than MOR-irrigated materials ($P = 0.004$) and maximum CO_2 evolution occurred at this time for both water sources and soil materials.

Discussion

Microbial Activity among Soil Materials and Water Sources

Total CO_2 evolution of preleached soil materials indicated that microbial activity was repressed in soil materials irrigated with WWE compared to those irrigated with MOR. The relatively greater soil salinity and sodicity, as measured by EC and ESP, respectively (Table 1), resulting from repeated irrigation with WWE, was probably responsible for suppressing soil microbial activity despite the favorable conditions of narrow carbon (C) / nitrogen (N) ratios and soil water content. Other studies also reported reduced microbial activity in saline soils or soils irrigated with wastewater effluents (Ghinogeanu, Stephanic, and Jonescu-Sisesti 1984; Mahasneh, Budour, and Doddema 1984; Garcia and Hernandez 1996; Pankhurst et al. 2001; Rietz and Haynes 2003).

The significant increase in CO_2 evolution immediately after leaching suggested that microbial activity responded to the leaching treatment, which decreased soil EC by 69% and 93% and ESP by 61% and 47% in the MOR- and WWE-irrigated soil materials, respectively. Decreasing salinity and ESP of soil materials produced a lower osmotic gradient between soil and microorganisms, which may have reduced osmotic stress on microbial activity (Schimel, Scott, and Killham 1989). Reduced osmotic stress may decrease immobilization of C and N in microbial biomass, thereby increasing C and N mineralization

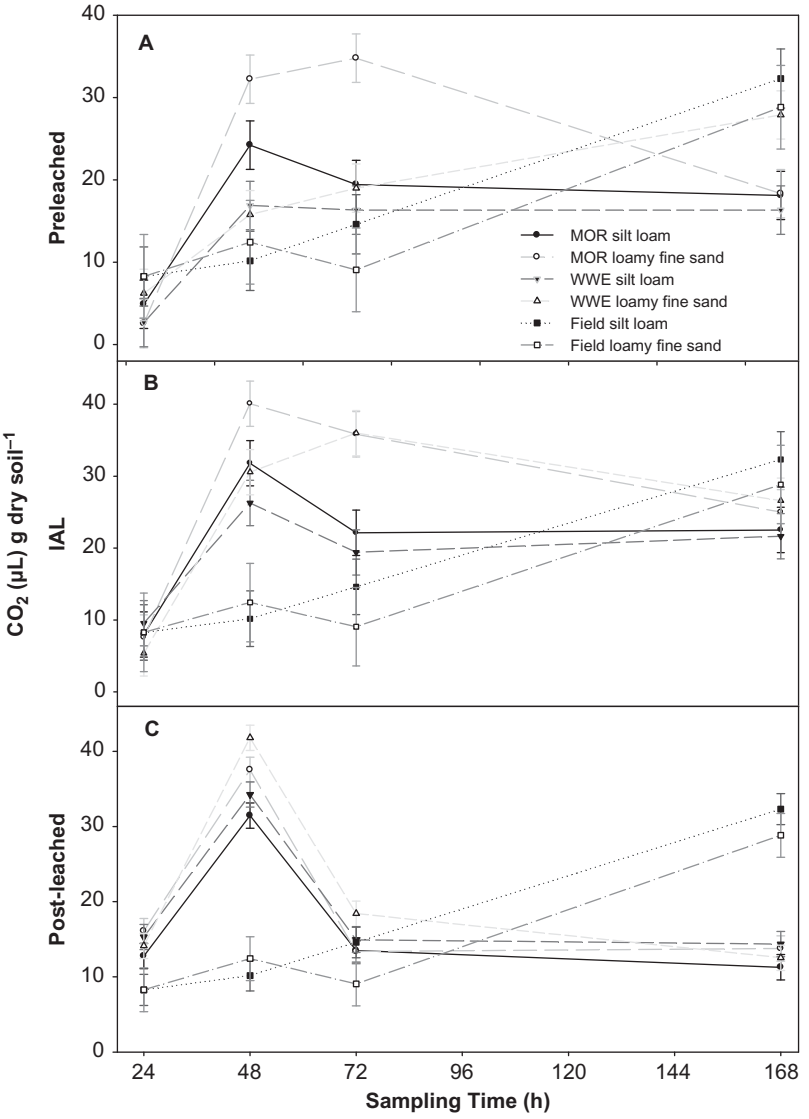

Figure 3. Mean total microbial CO_2 evolution by incubation time of soil materials collected from greenhouse microcosms before leaching (preleached), immediately after leaching (IAL), and after leaching and harvesting vegetation (postleached) that were irrigated with Missouri River (MOR) or municipal wastewater effluent (WWE). Field-collected soils (field) were collected from a wetland impoundment at Eagle Bluffs Conservation Area. Vertical bars indicate SE of the mean.

(Sarig, Roberson, and Firestone 1993). Additionally, the decrease in soil salts may allow development of a greater functionally diverse microbial community (Pankhurst et al. 2001).

The decline in CO_2 evolution in postleached treatments indicated less microbial activity in nearly all treatments, probably due to increases in EC and ESP resulting from resumption of irrigation. However, net changes in CO_2 relative to preleached conditions favored WWE-irrigated treatments. Immediately after leaching, soil materials irrigated with WWE had a greater net increase in evolved CO_2 (average of both soil materials)

than those irrigated with MOR. WWE-irrigated soil materials had an average increase of 27.4 μL CO_2 g dry soil^{-1} at IAL compared with 19.3 μL CO_2 g dry soil^{-1} in MOR-irrigated soil materials. Furthermore, postleached WWE-irrigated soil materials had a smaller net decrease in CO_2 evolution (4.9 μL CO_2 g dry soil^{-1}) than MOR-irrigated soil materials (20.2 μL CO_2 g dry soil^{-1}). The greater net gain and smaller net loss in evolved CO_2 at these sampling periods in the WWE-irrigated soil materials may be related to a combination of decreased salinity, utilization of residual labile soil C, and greater plant density and biomass that established after leaching. Sodicity has been reported to solublilize labile and recalcitrant organic materials; however, its effect can be hindered by salinity and anaerobisis (Abdou 1975; Nelson, Ladd, and Oades 1996). Labile C sources may have remained prior to and during the preleached trial when high salinity impaired the ability of sodium (Na) to solubilize C sources and repressed microbial activity. After leaching, C sources could be subject to mineralization under decreased salinity and reactivation of microbial communities as osmotic pressure decreased.

Labile C may also be derived from release of intracellular contents (i.e., amino acids, sugars) of lysed microbial cells, resulting from wetting (i.e., flushing) and drying (i.e., draining) cycles during the leaching treatment (Lund and Goksøyr 1980; Fierer, Schimel, and Holden 2002). Even though wet/dry cycles (resulting from periods in between irrigation applications) occurred during all trials, salinity was considerably greater during the preleached trial and continued to suppress microbial activity. In addition, root exudates, which increased as plants were established during the postleached trial, probably contributed to labile C sources.

Carbon Dioxide Evolution by Incubation Time

Peak respiration tends to occur at 48 to 72 h during laboratory incubations (Lund and Goksøyr 1980). In this study, maximum CO_2 evolution from MOR- and WWE-irrigated silt loam occurred at 48 h for each sampling period. Maximum CO_2 evolution from loamy fine sand of both water sources shifted to 48 h after leaching. In contrast, maximum CO_2 evolution of field-collected soil materials occurred at the 7-d sampling.

The CO_2 evolution in the loamy-fine sand microcosms occurring at 48 h may indicate a change in the microbial community from slow-responding, oligotrophic microorganisms to fast-responding copiotrophic microorganisms as more readily metabolizable C became available (Fierer, Bradford, and Jackson 2007). This shift could also be in response to reduced salinity due to leaching and the wet/dry cycles, which may favor copiotrophic microbial groups capable of rapid growth (Fierer, Schimel, and Holden 2002). Field-collected soil materials, on the other hand, which were not subjected to leaching, may have contained more recalcitrant C sources or perhaps more oligotrophic microorganisms. Field-collected soils contained greater concentrations of exchangeable Ca than soil materials in microcosms at the time of these samplings (Finocchiaro, unpublished data). Greater exchangeable Ca in the field-collected soil materials may have hindered microbial activity during the initial incubation (24 h), and activity increased after Ca linkages were disposed of (Nelson, Ladd, and Oades 1996). Another possibility is oligotrophic microbial groups, which respond slowly to substrate additions (i.e., glucose; Fierer, Bradford, and Jackson 2007), were dominant in the field-collected soil materials. This suggests that a shift in microorganism groups occurred in microcosms from oligotrophic to copiotrophic strategy. Hirsch et al. (1979) and Gottschal (1985) have reported that soil bacteria can switch from one strategy to another depending on environmental conditions and life stage.

Conclusion

Microbial activity was significantly impaired in soil materials irrigated with WWE compared with MOR as a water source. The greater EC and Na concentration of the WWE increased soil salinity and sodicity, which is thought to have inhibited microbial activity. After reducing soil EC and ESP by leaching, activity increased and was similar between water sources. A shift from oligotrophic to copiotrophic microbial groups may occur in the loamy-fine sand for both water sources in response to leaching of soil salts. Because soil microorganisms are critical to many soil processes such as nutrient cycling, aeration, aggregate development, and stability, decreased microbial activity may affect the plant community and other biotic systems, thereby negatively impacting these processes. Our results agree with those of Pankhurst et al. (2001) and Zahran (1997), who concluded that microbial community function may be affected by salinity only when the salt is actually present in soil and recovers when the salt is leached from the soil.

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